PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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05. Nov. 2004
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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	04.11.2004
Applicant's or agent's file reference G1184 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/09223	20.08.2003	20.08.2002

Applicant
B.R.A.I.N. BIOTECHNOLOGY RESEARCH AND..et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	de Haas, B Tel. +31 70 340-4738

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G1184 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09223	International filing date *(day/month/year)* 20.08.2003	Priority date *(day/month/year)* 20.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant B.R.A.I.N. BIOTECHNOLOGY RESEARCH AND..et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.01.2004	04.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Piret, B Telephone No. +31 70 340-1966

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09223

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-55 as originally filed

Sequence listings part of the description, Pages

1-65 as originally filed

Claims, Numbers

1-37 received on 06.10.2004 with letter of 06.10.2004

Drawings, Sheets

1/13-13/13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 27-37

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 27-37

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	1-24
	No:	Claims	25-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	none

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 99 00168 A (BIOSEPRA INC) 7 January 1999 (1999-01-07)

D2: MACNEIL I A ET AL: "EXPRESSION AND ISOLATION OF ANTIMICROBIAL SMALL MOLECULES FROM SOIL DNA LIBRARIES" JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, KOREAN SOCIETY FOR APPLIED MICROBIOLOGY, SEOUL, KO, vol. 3, no. 2, April 2001 (2001-04), pages 301-308, XP001050545 ISSN: 1017-7825

D3: BÉJÀ O ET AL: "Construction and analysis of bacterial artificial chromosome libraries from a marine microbial assemblage." ENVIRONMENTAL MICROBIOLOGY. ENGLAND OCT 2000, vol. 2, no. 5, October 2000 (2000-10), pages 516-529, XP002277482 ISSN: 1462-2912

D4: QUAISER ACHIM ET AL: "First insight into the genome of an uncultivated crenarchaeote from soil." ENVIRONMENTAL MICROBIOLOGY. ENGLAND OCT 2002, vol. 4, no. 10, October 2002 (2002-10), pages 603-611, XP002266613 ISSN: 1462-2912

1. Novelty (Article 33(1) and (2) PCT)

1.1. No device for the isolation/purification of nucleic acids and consisting of a two-phase gel, wherein the first phase is adapted to bind or inactivate inhibitors of reagents/enzymes used for DNA engineering and wherein the second layer is adapted to separate nucleic acids according to their size, could be found in the prior art. Therefore claims 1-24 meet the requirements of Article 33(2) PCT.

1.2. The subject-matter of claims 25-26 relates to gene libraries from planctonic or non-planctonic micro-organisms characterized by the average insert size and the (micro-organism) source. No such library could be found in the prior art and therefore claims 25-26 meet the requirements of Article 33(2) PCT.

1.3. This Preliminary Examination Report has been established after considering the priority application EP02018210 filed on 20.08.2002. Therefore, document D4 cited in the International Search Report is not considered as prior art for establishing the novelty/inventive step of the claimed subject-matter (Rule 64.1

PCT).

2. Inventive step (Article 33(1) and (3) PCT)

2.1. D1, which is the closest prior art, discloses a device for the isolation/ purification of nucleic acids comprising two layers. The subject-matter of claim 1 differs from the device of D1 by the fact that it comprises a two-phase gel, where one phase is adapted to bind and/or inactivate contaminants (enzyme inhibitors).

2.2. The problem addressed by the application can be defined as the provision of a further device having a layer adapted to remove contaminants. The solution proposed is a two-phase electrophoresis gel (instead of a chromatography column with two superposed media). Since this solution was suggested nowhere in the prior art, claims 1-24 meet the requirements of Article 33(3) PCT.

2.3. For claims 25 and 26, the closest prior art documents are D2 and D3, which disclose the isolation of genomic DNA from soil and planctonic microorganisms, respectively. In D2, genomic DNA from soil microorganisms could be isolated with a size ranging from 50 to 200 kB and could be efficiently digested with restriction endonucleases (Figure 1; p.302, right-hand column). Following partial digestion and sizing, inserts having sizes ranging from 5 to 120 kB were obtained. Similarly, D3 discloses the isolation of genomic DNA in fragments of >1 MB from marine microbes, its partial digestion, the isolation of fragments ranging from 150 to 300 kB in size, and the generation of a library from said fragments, with insert sizes ranging from 18 to 155 kB (p.517, right-hand column). The average insert sizes were respectively 37 kB in D2 and 60 or 80 kB in D3. The gene libraries of claims 25 and 26 differ from those of D2 and D3 by the average size of the inserts. The problem addressed by the application is therefore: the provision of DNA libraries from planctonic or non-planctonic micro-organisms with an increased average insert size. The solution proposed by the applicant consists of a DNA library obtained from high molecular weight genomic DNA isolated and purified using the device of claim 1.

2.4. The factors affecting the average insert size in the library appear to be the quality ("digestibility") and the size of the genomic DNA, as well as the average size and the dispersion of the size of the digested fragments prior to cloning in the library. From the data provided in the application (p.39 and beyond), it is not apparent that the method and device of the invention represent an improvement

over the prior art regarding these parameters, nor that only said method and device are suitable for obtaining libraries with an average size of at least 50 kB (claim 25) or 85 kB (claim 26). The methods used in the prior art (phenol extraction followed by treatment with CTAB and sucrose density gradient centrifugation in D2; direct lysis of agarose-embedded microorganisms in D3) seem as suitable for obtaining high molecular weight, pure (digestible) genomic DNA from environmental samples. It appears from D2 and D3 that it is obvious for a person skilled in the art to obtain libraries with a higher average insert size simply by selecting (by excision from the agarose gel) the largest digested fragments (e.g. the fragments having a size of 250 to 300 kB rather than 150 to 300 kB in D2). Furthermore, the available evidence does not indicate that the invention actually solves the problem of obtaining libraries with average fragment sizes as high as 100 kB for non-planctonic sources (claim 25) or as high as 200 kB for planctonic sources (claim 26). Therefore the subject-matter of claims 25-26 does not meet the requirements of Article 33(3) PCT.

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CLAIMS

1. A device for the isolation and/or purification of nucleic acid molecules comprising at least two layers, a first layer being adapted to bind and/or inactivate inhibitors of the activity of reagents or enzymes used in nucleic acid manipulation and a second layer being adapted to separate a plurality of nucleic acid molecules with respect to their size and wherein said first layer is a first phase of a gel and said second layer is a second phase of said gel.

2. The device of claim 1, wherein said first layer is arranged above the second layer.

3. The device of claim 1 or 2, wherein said gel is an agarose-gel or a polyacrylamid-gel.

4. The device of any one of claims 1 to 3, wherein said first layer comprises polyvinylpyrrolidone (PVP), polyvinylpolypyrrolidone (PVPP), CTAB, EDTA, EGTA, cyclodextrins, proteins, (poly)peptides, antibodies, aptamers, lectins, nucleic acids or ion-exchanger.

5. The device of any one of claims 1 to 4, wherein said second layer is substantially free of PVP, PVPP, CTAB, EDTA, EGTA, cyclodextrins, proteins, (poly)peptides, aptamers, antibodies, lectins, nucleic acids or ion-exchanger.

6. The device of any one of claims 1 to 5, wherein the device is electrically biased to enhance flow of (a) sample(s) through the layers.

7. The device of any one of claims 1 to 6, wherein said first layer comprises sample loading means.

8. The device of claim 7, wherein said loading means are provided in an array in an upper portion of the first layer, defining an array of columns, each being capable of isolating nucleic acid molecules.

9. The device of any one of claims 1, 4, 5, 6, 7 or 8, wherein said first layer is arranged below the second layer.

10. The device of claim 9, which is a column comprising said first and said second layer.

11. The device of claim 9 or 10, wherein said second layer is a first phase of a column and said first layer is a second phase of said column.

12. The device of any one of claims 9 to 11, said first layer is a matrix comprising PVP, PVPP, CTAB, EDTA, EGTA, cyclodextrins, proteins, (poly)peptides, aptamers, antibodies, lectins, nucleic acids or ion-exchanger.

13. The device of any one of claims 9 to 12, wherein said second layer is a matrix which is substantially free of PVPP PVP, PVPP, CTAB, EDTA, EGTA, cyclodextrins, proteins, (poly)peptides, aptamers, antibodies, lectins, nucleic acids or ion-exchanger.

14. The device of claims 12 or 13, wherein said matrix of said first and/or second layer is selected from the group consisting of agarose, sepharoseTM, sephadexTM, sephacrylTM, BioGelTM, superoseTM and acrylamid.

15. The device of any one of claims 1 to 14, wherein said nucleic acid molecule is DNA or RNA.

16. The device of claim 15, wherein said DNA is genomic DNA.

17. The device of claim 15 or 16, wherein said nucleic acid molecule is derived from (micro)organisms of soil, sediments, water or symbiotic/parasitic consortia.

18. The device of claim 17, wherein said (micro)organisms are (micro)organisms of aquatic plancton, microbial mats, clusters, sludge flocs, or biofilms.

19. The device of claim 17 or 18, wherein said (micro)organism are isolated as consortia of coexisting species.

20. The device of any one of claims 1 to 19, wherein said nucleic acid molecules represent a fraction of the metagenome of a given habitat.

21. A method for the isolation of a nucleic acid molecule comprising applying a sample to the device as defined in any one of claims 1 to 20.

22. The method of claim 21, wherein a fraction of the metagenome is isolated from a given habitat.

23. A method for the generation of at least one gene library, comprising the steps of
    (a) isolating and/or purifying nucleic acid molecules from a sample using a device as defined in any one of claims 1 to 20 and optionally amplifying said nucleic acid molecules;
    (b) cloning the isolated and/or purified and optionally amplified nucleic acid molecules into appropriate vectors; and
    (c) transforming suitable hosts with said suitable vectors.

24. The method of claim 23, wherein said suitable hosts are selected from the group consisting of E. coli, Pseudomonas sp., Bacillus sp, Streptomyces sp, other actinomycetes, myxobacteria, yeasts and filamentous fungi.

25. A gene library generated from metagenomic nucleic acid molecules from non-planctonic (micro)organisms comprising average insert sizes of at least 50 kB, of at least 55 kB, of at least 60 kB, of at least 80 kB, of at least 90 kB or of at least 100 kB.

26. A gene library generated from metagenomic nucleic acid molecules from planctonic (micro)organisms comprising average insert sizes of at least 85 kB, at least 90 kB, at least 95 kB, at least 100 kB, at least 120 kB, at least 140 kB, at least 160 kB or at least 200 kB.

27. A nucleic acid molecule comprising a DNA as depicted in SEQ ID NO: 1 or a DNA as deposited under EMBL accession number A3496176.

28. A nucleic acid molecule representing a part of the genome of a non-thermophilic crenarchaeota, whereby said nucleic acid molecule has at least one of the following features:

(a) it contains at least one ORF which encodes a polypeptide having the amino acid sequence SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35;

(b) comprises the DNA sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34;

(c) it comprises portion of at least 20 nucleotides, preferably 100 nucleotides, more preferably at least 500 nucleotides which hybridise under stringent conditions to the complementary strand of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID

NO: 32, SEQ ID NO: 34;

(d) it is degenerate as a result of the genetic code with respect to the nucleic acid molecule of (c); or

(e) it is at least 50% identical with the nucleic acid molecule of SEQ ID NO: 2, SEQ ID NO: 20 or SEQ ID NO: 30, 45% identical with the nucleic acid molecule of SEQ ID NO: 8 or SEQ ID NO: 26, 35% identical with the nucleic acid molecule of SEQ ID NO: 16, SEQ ID NO: 22 or SEQ ID NO: 24 or 30% identical with the nucleic acid molecule of SEQ ID NO: 4, SEQ ID NO: 14, SEQ ID NO: 18 or SEQ ID NO: 28;

29. The nucleic acid molecule of claim 27 or 28, which is DNA or RNA.

30. A vector comprising the nucleotide acid molecule of any one of claims 27 to 29.

31. A host transfected or transformed with the vector of claim 30.

32. A method for producing a (poly)peptide as encoded by a nucleic acid molecule of any one of claims 27 to 29, comprising culturing the host of claim 31 under suitable conditions and isolating said polypeptide from the culture.

33. A (poly)peptide encoded by a nucleic acid molecule of any one of claims 27 to 29 or as obtained by the method of claim 33.

34. The (poly)peptide of claim 33 which is glycosylated, phosphorylated, amidated and/or myristylated.

35. An antibody or an aptamer specifically recognizing the (poly)peptide of claim 33 or 34 or a fragment or epitope thereof.

36. The antibody of claim 35, which is a monoclonal antibody.

37. A transgenic non-human mammal whose somatic and germ cells comprise at least one gene encoding a functional polypeptide selected from the group consisting of:

(a) the polypeptide of claim 33 or 34;

(b) a polypeptide having an amino acid sequence that is at least 60%, preferably at least 80%, especially at least 90%, advantageously at least 99% identical to the amino acid sequence of (a); and

(c) a polypeptide having the amino acid sequence of (a) with at least one conservative amino acid substitution.